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HIV-1 Transgenic Rat: Selective Alterations In Motivation And Histological Examination Of Medium Spiny Neurons Of The Nucleus Accumbens

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by

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DEDICATION

I would like to dedicate this thesis in memory of my mother Julie and fellow graduate student Alex P. Ojeda; both of whom were taken from the world before their time. Both always pushed me to achieve the best I could and never give up on my dreams, it is in their memory that I dedicate this work. Additionally, I am dedicating the current work to my graduate mentors: Dr. Rosemarie Booze, Dr. Charles Mactutus, and Dr. Steven Harrod. It is because of their undying faith in my abilities, funding and advice that this project was possible. With their guidance and advice, the quality of the current study was constantly improved. They forever have my respect and admiration. Thank you all for your respective roles in my education.

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ABSTRACT

Motivational alterations in HIV-1+ individuals are associated with decreased performance on tasks involving frontal-subcortical circuitry and the nucleus accumbens. In the present study, the HIV-1 transgenic (Tg) rat was used to assess long-term HIV-1 viral protein exposure on motivated behavior using activity chambers (40x40cm) and voluntary wheel running. Adult ovariectomized female HIV-1 Tg animals (n=21) to F344 controls (n=26) were pair-housed under a 12:12 light/dark cycle. Voluntary running was measured with 34 cm-diameter running wheels for ~60 minutes/day for 3 ½ months. There were no significant differences between HIV-1 Tg and F344 control rats in voluntary wheel running during the light phase. Animals were subsequently run in the nocturnal phase of their light/dark cycle. The F344 controls continued to escalate their overall running distances and surpassed the stabilized HIV-1 Tg group after ~4 weeks of nocturnal running, until reaching their asymptotic plateau at week 11. Neither maximal running speed, nor the latency to initiate running or running bout length were significantly different between groups. However, the groups were different in the number of running bouts per session, as a function of the HIV-1 transgene. Collectively, the selective alterations in the motivation for voluntary wheel running and activity chamber locomotor activity, suggests a disruption of the motivational circuitry within the HIV-1 Tg rat brain. Examination of Medium Spiny Neurons of

the nucleus accumbens showed significant alterations in dendritic spine length and spine head diameter. Further study of these alterations in spine parameters may help elucidate the mechanisms of motivational alterations in HIV-1+individuals.

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LIST OF SYMBOLS

X^2 ..... Chi-Square analysis

LX^2 ..... Chi-Square analysis; Maximum Likelihood Ratio reported

LIST OF ABBREVIATIONS

ANOVA ..... Analysis of Varianc

ART ..... Antiretroviral Therapies

AMPA ..... α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

cART ..... Combined Antiretroviral Therapies

CaMKII ..... Ca^{2+}/Calmodulin-dependent Protein Kinase

CNS ..... Central Nervous System

DAT ..... Dopamine Transporter

EPSP ..... Excitatory Postsynaptic Potential

HAND ..... HIV-1 Associated Neurocognitive Disorder

HIV-1 ..... Human Immunodeficiency Virus, first subtype

IACUC ..... Institutional Animal Care and Use Committee

LTD ..... Long-term Depression

LTP ..... Long-term Potentiation

mPFC ..... medial Prefrontal Cortex

MSN ..... Medium Spiny Neuron

NAc ..... Nucleus Accumbens

NIH ..... National Institutes of Health

NMDA ..... N-methyl-D-aspartate receptor

p_{GG} ..... Greenhouse-Geisser Adjustment incorporated

PKC ..... Protein Kinase C

PND .....Postnatal Day

PVP..... Ppolyvinylpyrrolidone

Rac1 ..... Ras-related C3 botulinum toxin substrate 1

RhoA..... Ras homolog gene family, member A

Tg..... Transgenic

VTA..... Ventral Tegmental Area

WT .....Wild-Type

CHAPTER 1

INTRODUCTION

HIV-1 infection continues to increase in global prevalence despite continued public education regarding HIV-1. As of 2015, approximately 37 million individuals worldwide were infected with the virus, according to the World Health Organization (World Health Organization, 2015). Since the advent of anti-retroviral therapies (ART), virally-mediated mortality and quality-of-life has been improved in seropositive individuals. Yet, despite the improvement in disease pathology, the prevalence of the HIV-associated-neurocognitive disorders (HAND) has remained stable globally throughout the pre-ART and post-ART eras (Heaton et al., 2010; Heaton et al., 2011; Heaton et al., 2015; McArthur, Steiner, Sacktor, & Nath, 2010; Watkins & Treisman, 2015). Within the realm of cognitive impairments, reduced motivation is prevalent in ~50% of HIV-1 seropositive individuals (Castellon, Hinkin, & Myers, 2000; Kamat et al., 2015; Tate et al., 2003).

Reduced motivation (apathy) in HIV-1 infected individuals can significantly affect perceived well-being and depression (Tate et al., 2003), medication adherence (Barclay et al., 2007; Panos et al., 2014), attentional tasks, executive function and reward-cue processing (Plessis et al., 2015). Apathy is prevalent in the HIV-1 population with estimates of 30-50% of participants affected (Castellon et al., 2000; Kamat et al., 2015; Tate et al., 2003). Apathy and self-efficacy were

found to be the strongest psychosocial predictors of medication adherence in HIV-1 patients who were taking combination antiretroviral therapies (cART) (Panos et al., 2014). Yet, despite the prevalence of apathy in the HIV-1+ population, reductions in motivation is understudied. The goal of the present study was to examine apathy in the HIV-1 population in a pre-clinical animal model, the HIV-1 Transgenic (Tg) rat.

Motivation, or the lack thereof (apathy) in HIV-1 patients, may be due to basal ganglia atrophy, which is apparent in HIV-1 seropositive individuals through brain imaging techniques (Paul et al., 2005). Basal ganglia atrophy is associated with reduced motivation in the HIV-1 population (McIntosh, Rosselli, Uddin, & Antoni, 2015). These findings suggest the dopamine system in HIV-1 patients is altered by the HIV-1 associated neurotoxic proteins. In the majority of pertinent literature (Castellon et al., 2000; Cole et al., 2007; Kamat, Woods, Marcotte, Ellis, & Grant, 2012; Kamat et al., 2015; Levy & Dubois, 2006; Paul et al., 2005), apathy carries a shared definition, and was defined initially by Marin (1991) as: “a syndrome of primary motivational loss, that is a loss of motivation not attributable to emotional distress, intellectual impairment, or diminished level of consciousness” (Marin, 1991).

The HIV-1 Tg rat is one animal model for the study of HIV-1 disease progression and neurocognitive alterations (Moran, Aksenov, Booze, Webb, & Mactutus, 2012; Moran, Booze, Webb, & Mactutus, 2013). Of the 9 genes encoded by the HIV-1 virus, 7 are expressed in the HIV-1 Tg rat. The genetic alterations which occur in HIV-1 patients, and the aviremic state of patients

whom are on cART (Peng et al., 2010; Vigorito, Connaghan, & Chang, 2015) can be replicated by the HIV-1 Tg rat animal model. The *pol* protein (1 of the 2 non-expressed proteins) is utilized in transcription and replication of the HIV-1 virus; therefore an aviremic state is replicated in the HIV-1 Tg rat. The neurotoxic proteins found within the central nervous system (CNS) continue to be expressed in the HIV-1Tg rat, similar to what is found in human HIV-1+ individuals (Brew et al., 2015; Peng et al., 2010; Rappaport & Volsky, 2015) adhering to cART.

The HIV-1 Tg rat was developed through embryonic delivery of a non-infectious provirus, with the *gag* and *pol* genes deleted. The transgene was located on one allele in the initial female founder (hemizygous). The animal was bred with a wild-type (WT) inbred F344; pups with either the HIV-1 transgene (HIV-1 Tg) or wild type (Tg-WT) were created from the breeding combination. The HIV-1 transgene is incorporated into all cells (20-25 copies) of the HIV-1 Tg rat, causing opaque cataracts (Reid et al., 2001). The viral gene *env* (envelope protein), is an essential gene for the HIV-1 provirus to attach to the lipid bilayer of the host cell (macrophages and CD4+ T cells), is coded by the transgene. The gp160 precursor is coded by the *env* protein, and then is cleaved into gp120 and gp41 (McCune et al., 1988) which collectively allow the virus to bind to the CD4 glycoprotein or chemokine (CCR5 or CXCR4) receptors on the host cell's surface (via gp120) and deliver its viral contents (via trimeric gp41) (Chan & Kim, 1998; Wyatt & Sodroski, 1998). While the HIV-1 associated proteins (*env, tat, rev, vif, vpf, vpu* and *nef*) are produced in the animal model (Peng et al., 2010; Reid et

al., 2001), the HIV-1 Tg animal model is not suitable to examine the viral replication or transmission stages (due to the *pol* and *gag* deletions).

However, the HIV-1 Tg rat is ideal for examination of neurocognitive alteration in individuals who adhere to cART. As stated previously, 7/9 of the HIV-1 viral proteins are expressed by the HIV-1 transgene throughout development and into adulthood and old age (Abbondanzo & Chang, 2014). The HIV-1 Tg rat provides a tool for examination of HIV-1 neural alterations through continued expression of the HIV-1 associated viral proteins. The HIV-1 Tg rat is a promising model for examination of potential theraputics for HIV-1 cognitive progressive decline and, as hypothesized, motivational deficiencies. Thus far, the HIV-1 Tg rat has been used to study neurological deficiencies (Moran et al., 2013; Moran, Booze, & Mactutus, 2013; Moran, Booze, & Mactutus, 2014; Peng et al., 2010; Royal, III, Wang, Jones, Tran, & Bryant, 2007; Royal, III et al., 2012; Vigorito et al., 2015), sensorimotor gating (Moran et al., 2013; Moran et al., 2013; Moran, Hord, Booze, Harrod, & Mactutus, 2015), attention and inhibition (Moran et al., 2014), and medium spiny neuron (MSN) dendritic spine alterations (Roscoe, Jr., Mactutus, & Booze, 2014).

Significant reductions in locomotor activity (June, Tzeng Yang, Bryant, Jones, & Royal, III, 2009; Midde, Gomez, Harrod, & Zhu, 2011; Moran et al., 2013) as well as differential rates of habituation to the activity apparatus (Moran et al., 2013) compared to controls, have been previously reported in the HIV-1 Tg rat. These findings suggest the motivation for exploration or escape may be reduced in the HIV-1 Tg rat. The purpose of the present study was to examine

apathy and reduced motivation in the HIV-1 Tg rat. It was hypothesized the HIV-1 Tg rat would exhibit reduced intrinsic motivation compared to age-matched controls.

Research in human seropositive individuals has shown the circadian rhythms of HIV-1 patients is altered, with 50-70% reporting disturbances in sleep onset (insomnia), daytime sleepiness, and fragmented sleep during standard sleeping hours. Altered circadian rhythms (reduced amplitudes) has been shown to be directly caused by the Transactivator of Transcription (Tat) protein (Clark, III, Sampair, Kofuji, Nath, & Ding, 2005; Duncan et al., 2008; Wang et al., 2014), and the altered circadian rhythmicity has been found in both human (Wang et al., 2014) and animal models (Clark, III et al., 2005; Huitron-Resendiz, Marcondes, Flynn, Lanigan, & Fox, 2007). The goal was to determine if chronic expression of the Tat protein affects the capacity for entrainment to new or altered light and dark cycles. It has been previously reported, Tat is capable of altering the established circadian cycle-induced animal metabolic frequency amplitudes in models of HIV-1 progression (reduced circadian rhythm amplitude) (Duncan et al., 2008; Huitron-Resendiz et al., 2007). As alterations in circadian patterns may affect diurnal/nocturnal running behaviors differentially, circadian influence on the HIV-1 Tg rat in relation to motivation was examined. Alterations in the circadian rhythms of the HIV-1 Tg rat would be elucidated by the voluntary wheel running task and experimental time-of-day manipulations if they were apparent was the goal of the current study.

Motivated behaviors in rats, such as area exploration and wheel running, are dependent upon ventral tegmental area (VTA) dopaminergic activation of the nucleus accumbens (NAc) →medial prefrontal cortex (mPFC) (mesocorticolimbic dopaminergic pathway) (Basso & Morrell, 2015; Greenwood et al., 2011; Mogenson, Wu, & Manchanda, 1979; Mogenson & Yang, 1991; Pijnenburg, Honig, Van der Heyden, & Van Rossum, 1976; Rhodes, Gammie, & Garland, Jr., 2005; Roberts et al., 2012). Previous research has shown when the dopaminergic activity of mesocorticolimbic pathway is upregulated (SKF82958, methylphenidate (Ritalin™), cocaine, GBR 12909) or downregulated (SCH23390, Bupivacaine, and muscimol) through pharmacotherapies, the motivation for running is increased or decreased (respectively) in both the acquisition and habitual phases of wheel running (Basso & Morrell, 2015; Rhodes et al., 2005; Roberts et al., 2012). Additionally, when animals are forced to abstain from the running wheels (deprivation), upon running wheel return the animals show a "rebound effect" (i.e. increase) in their running distances (Basso & Morrell, 2015; Mueller, Herman, & Eikelboom, 1999; Skinner B.F., 1933). The rebound effect in wheel running suggests the activity has rewarding properties for the animals, similar to those found after deprivation of other naturally rewarding stimuli (Heffner, Hartman, & Seiden, 1980), or pharmacological (drug) rewards (McSweeney, Murphy, & Kowal, 2005). Voluntary wheel running was a free-choice activity for the animals, therefore it was hypothesized that wheel running was a task capable of assessing intrinsic motivation in the HIV-1 Tg rat for a naturally rewarding stimulus.

Within the mesocorticolimbic pathway, MSNs of the NAc act as inhibitory (GABAergic) regulators of the dopaminergic efferent projections from the VTA, and the glutamatergic efferent projections from the mPFC (Purves et al., 2012a; Purves et al., 2012b). As the MSNs of the NAc are structurally altered in the HIV-1 Tg rat (Roscoe, Jr. et al., 2014), it is possible the motivational state of the HIV-1 Tg rat is effected due to neural alterations of the NAc. Neuronal synapses and dendritic spines are subject to increased dendritic loss and aberrant sprouting of synaptodendritic connections and reduced plasticity in HIV-1 patients; for review see (Avdoshina, Bachis, & Mocchetti, 2013; Ellis, Langford, & Masliah, 2007). MSNs of the nucleus accumbens are structurally altered in the HIV-1 Tg rat according to previous reports (Roscoe, Jr. et al., 2014). The motivational/rewarding efficacy of naturally rewarding behaviors may be effected by alterations of the mesocorticolimbic pathway (Purves et al., 2012a). As the nucleus accumbens is sensitive to and targeted by, HIV-1 neural proteins resulting in neural alterations, it was paramount to histologically examine these areas. Specifically, the nucleus accumbens, and the inhibitory MSNs it contains, are an essential feature of the subcortical circuitry of the reward/motivation pathway. As voluntary wheel running requires mesocorticolimbic activation, histological examination of the MSNs of the NAc was conducted. Previously, it has been reported the HIV-1 Tg rat has altered dendritic spines on the MSNs of the NAc, with significant alterations in dendritic spine length and volume measures (Roscoe, Jr. et al., 2014) compared to controls. Consequently,

motivated behaviors such as voluntary wheel running may be impacted by alterations in MSN dendritic spine morphology.

In summary, motivation in the HIV-1 Tg rat was tested using locomotor activity in an activity chamber and a voluntary running wheel. To determine if there are any motivational differences due to a genotype × diurnal phase interaction, diurnal influences were compared between the HIV-1 Tg rat and F344 controls. These manipulations were done to assess the effect of HIV-1 on circadian rhythmicity of voluntary wheel running in the HIV-1 Tg rat compared to age-matched controls. Further insight into motivation deficiencies was provided by examination of the MSNs of the NAc. Altered synaptodendritic connections in the reward/motivation pathway (mesocorticolimbic) were due to MSN alterations in dendritic spine parameters, which behaviorally created a reduced motivational state in the HIV-1 Tg rat. To date, there are no publications on the MSNs of the NAc immediately following a behavioral task in the HIV-1 Tg rat.

CHAPTER 2

METHODS

2.1 SUBJECTS

Ovariectomized female Fisher (F344/N; Harlan Laboratories Inc., Indianapolis, IN) rats (F344 controls, *n*=26 and HIV-1 Tg, *n*=21) arrived at the facility at postnatal day (PND) 46-60 (1 ½-2 months of age). All animals were pair-housed throughout the experiments and randomly assigned numbers for identification and wheel apparatus assignment. Rodent food (2020X Tekland Global Soy Protein-Free Extruded Rodent Diet (Soy Protein-Free)) and water were available *ad libitum* for the entirety of the study. Animals were maintained according to the National Institutes of Health (NIH) guidelines in AAALAC-accredited facilities. The animal facility was maintained at 21° ±2° Celsius, 50% ± 10% humidity and had a 12-hour (h) light/12h dark cycle with the lights on at 0700h (EST). The project protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of South Carolina, animal assurance number A3049-01. An overview of the experimental testing sequence is shown in Table 2.1.

2.2 BODY WEIGHTS:

All animals were handled and weighed 5 days per week from the day they arrived at the facility (PND 46-60) for the duration of the study to observe animal

overall health on a digital scale, with weights to the tenth of a gram (Mettler Toledo, Columbus, OH).

2.3 LOCOMOTOR ACTIVITY

2.3.1 Apparatus

Animals were tested in activity chambers (40cm x 40cm) fitted with a circular Plexiglas insert to promote animal movement and prevent animals from stopping in the corners of the apparatus (Hughes & Beveridge, 1986; Hughes, Lowther, & van, 2011; Moran et al., 2013). Ambulation and animal rearing in the X and Y axis was recorded via infrared photocells spaced 2.5cm apart (Hamilton-Kinder Inc., Ponway, CA). Photocell breaks were recorded by Digipro Systems Software (v.140, AccuScan Instruments). The dependent measures of gross, fine, and rearing movements were defined by the monitoring software Motor Monitor (Hamilton-Kinder Inc., Ponway, CA). Locomotor activity was recorded and grouped in 5-minute bins. Gross movement was classified as clearing of the anchor beam when a new beam was broken, while fine movements (such as head movements) were defined by new beam breakage without clearance of the anchor beam. Photocell pairs were tuned by the manufacturer to control for extra perspex width due to the presence of the circular Plexiglas insert (~40cm in diameter).

2.3.2 Procedure (Phase I): Locomotor activity

Activity testing was performed once per week for seven consecutive weeks. Animals were placed in the center of the activity chamber and recording

initiated. Animals were tested from 1200h to 1600h under low-light conditions (10 Lux). White noise of 70db was played in the testing room for the duration of the activity measure (60 minutes). Animal testing was block-randomized through Latin Square design for testing session and chamber assignment to control for time of day and box proximity to the light/sound source.

2.4 RUNNING WHEELS

2.4.1 Apparatus

The running wheel apparatus was a polypropylene rectangular chamber (45.5cm long, 24cm wide and 20.5cm deep) with a 34cm diameter running wheel attached to the chamber lid at the midpoint. Magnetic sensors on the lid and wheel recorded revolutions by the recording software. All data was recorded by Vital View 4.0 (Mini Mitter Inc., Bend, OR) software which records wheel revolutions. The data was collected into 1-minute cumulative bins for wheel revolutions.

2.4.2 Diurnal Procedure (Phase II): Wheel Running

The animals were tested in voluntary wheel running in the diurnal phase of their light/dark cycle following open-field activity testing. Testing was performed from 12:00 to 17:00 each day for ~66 minutes, 7 days per week. Testing was continued until stability was reached in overall group running distance for 3 consecutive weeks (not significantly different). Animals were assigned to their running wheel chamber through Latin-Square design to ensure an equal chance

to be tested in any chamber or running group (time of day). Diurnal running sessions were performed in low-light conditions (10 lux). Following the wheel-running session, animals were returned to their home cage until the next session the subsequent day.

2.4.3 Nocturnal Procedure (Phase III): Wheel Running

Once diurnal stability in both groups was established, animals were then tested during the nocturnal phase of their light/dark cycle, with testing initiated at 19:00 and continuing until approximately 24:00, following the same procedure as diurnal running sessions. The animals were placed in the same designated wheel chamber as previously assigned and tested in the dark (<10 lux). Animals were run 7 days/week until running distances for both groups stabilized for 3 consecutive weeks.

2.4.4 Deprivation/Response trials (Phase IV): Wheel Running

Following 3 weeks of stabilized asymptotic nocturnal running performance, the running wheels were locked for either 1, 2, or 3 days to measure group responsiveness to varying locked wheel durations, and determine if the response was similar in both groups. The sequence of Phase IV is shown in Table 2.2.

2.4.5 Diurnal Return: Wheel Running

To determine if the increased running in the nocturnal sessions was a consequence of learning or conditioning, animals were returned to the diurnal schedule for one week following Phase IV (Deprivation/Response phase). It was hypothesized a return to distances previously established during diurnal recording (Phase II) would support the notion that the differences found between diurnal/nocturnal running sessions (Phase II & III) were a product of a well-entrained circadian cycle. Running distances between diurnal stabilized levels (Phase II) and nocturnal stabilized levels (Phase III) would suggest a learning or conditioning phenomenon had taken place.

2.4.6 Peak running speed: Wheel Running

Individual peak running speed was determined as: peak speed= 95^{th} percentile * Wheel circumference (106.76cm) (Mahoney et al., 2013; Ottenweller et al., 1998) during both diurnal and nocturnal running sessions (analyzed separately). Peak running speed was determined by the 95^{th} percentile of wheel revolutions during any 1-minute bin. Wheel running data (Phase II & III) was analyzed to determine individual and group average peak running speed. Individual peak speed data was analyzed for genotype differences.

2.4.7 Motivation for running initiation latency: Wheel Running

The latency for the groups to initiate their first revolution during diurnal and nocturnal running sessions (Phase II & III) was examined. Latency for initiation

was determined through individual data from each phase. Initiation latency was defined as: initiation latency = group average of the number of minutes from the start of the session until the first revolution. Diurnal and nocturnal initiation latency was analyzed separately.

2.4.8 Running Bout length and frequency: Wheel Running

Running bouts were determined by: number of minutes in which at least 1 wheel revolution occurred in sequence until no revolutions occurred (quiescent) (Eikelboom & Mills, 1988). Running bouts were analyzed for bout length and total number of bouts similar to previous studies (Eikelboom & Mills, 1988).

2.5 HISTOLOGY

2.5.1 Euthanasia Procedure

At the end of the study, all animals were tested one last time. At the end of ~66 minutes, the animals were removed from the wheel apparatus and immediately deeply anesthetized with sevoflurane and then transcardially perfused with ~100ml of 100mM PBS followed directly by ~150ml of chilled 4% paraformaldehyde buffered in PBS (Sigma-Aldrich, St. Louis, MO). Brains were removed and post-fixed in 4% chilled paraformaldehyde for 10 minutes, notched for orientation, then sectioned in 200µm thick coronal slices using the rat brain matrix (ASI Instruments, Warren, MI). Sequential slices were placed in a 24-well plate (Corning, Tewksbury, MA) with 1ml PBS until processed.

2.5.2 Preparing the DiOlistic cartridges

Tungsten beads (175mg, Bio-Rad, Hercules, CA) were dissolved in ~0.3ml of 99.5% methylene chloride (Sigma-Aldrich, St. Louis, MO). Crystallized DiI (7mg, Life Technologies, Grand Island, NY) was dissolved in ~0.3ml methylene chloride. All of the bead solution was applied to a glass slide and allowed to air dry completely. The DiI solution was added to the affixed tungsten beads on the glass slide and mixed slowly with the pipette tip until completely dried. A razor blade was used to scrape the dye/bead mixture into two (2) 1.5ml Eppendorf™ (Eppendorf North America, Hauppauge, NY) tubes with 1.5 ml of ddH2O water. These two tubes were probe sonicated with a Branson Sonifier 150 (Branson Ultrasonics; Danbury, CT) for a minimum of 10 minutes to ensure complete breakup of the beads which had adhered together by the DiI solution. The two tubes were then combined into a 15ml (BD Falcon, San Jose, California) and sonicated further until used later.

2.5.3 Preparation of Tefzel Tubing

First, ~70cm of new Tefzel tubing (IDEX Health Sciences, Oak Harbor, WA) was cut. Polyvinylpyrrolidone (PVP) (100 mg Sigma-Aldrich, St. Louis, MO) was dissolved into 10ml of ddH2O, vortexed, and drawn into the Tefzel tubing and allowed to sit for a minimum of 10 minutes. When ready, the PVP was removed from the tube and the DiOlistic mixture was drawn into the tube between periodic vortexes to maintain homogenization. The filled tubing was placed in the tubing prep station (Bio-Rad, Hercules, CA) and rotated for 5

minutes. Rotation was stopped momentarily while the remaining water was slowly drawn from the tubing, and rotation resumed for another 30 minutes. Nitrogen gas was added to ensure drying for another 30 minutes at a flow rate of 0.4 L/m. Once dry, the tubing was cut into 13mm segments and stored in a dark condition.

2.5.4 DiOlistic Labeling using the Helios Gene Gun

For labeling of the MSNs in the NAc (from Bregma 3.24mm-.48mm, plates 10-29 (Paxinos & Watson, 2007), slices were DiOlistically labeled using the Helios Gene Gun (Bio-Rad, Hercules, CA) following the initially reported procedure (Roscoe, Jr. et al., 2014; Seabold, Daunais, Rau, Grant, & Alvarez, 2010). Bullets were ballistically delivered once at ~90 PSI to the coronal slices, through a 3μm pore filter paper with the sample ~2.5cm away from the gun barrel, slices were washed 3x to ensure excess DiI coated tungsten bullets were removed from the wells. These slices were stored in PBS at 4° C in the dark overnight to allow dye diffusion before mounting.

2.5.5 Mounting of NAc slices and slide preparation

Following DiOlistic labeling, Slices 3, 4, & 5 from the rat brain matrix (approximately Bregma 2.28-1.00, (Paxinos & Watson, 2007); plates 14-25) were used for imaging of the NAc MSNs. Three slices per animal were placed on 25 x 75 x 1mm Superfrost Plus slides (VWR, Radnor, PA), completely covered using

ProLong Gold antifade reagent (Life Technologies, Grand Island, NY) and coverslipped with 22x50mm VWR micro cover glass (VWR, Radnor, PA).

2.5.6 Imaging and Spine Measurement

Slices were imaged with a Nikon TE-2000E confocal microscope, using Nikon's EZ-C1 computing software (v. 3.81b) to create Z-stacked images of the DiOlistically labeled neurons in the NAc and analyzed by dendritic spine analysis software. Z-stacks of the MSNs of the NAc were created to build 3-dimensional reconstructions of the neurons to measure the dendritic spines of the MSNs. The anterior commissure was used as a reference point to find the NAc area in each slice. These 3-dimensional images were analyzed using Neurolucida (v. 10.52) AutoNeuron and Autospine (MicroBrightField, Williston, VT) modules to measure the dendritic spine length and head diameter.

2.5.7 Dendritic Spine Parameters

Dendritic spine measurements of the MSN's of the NAc was quantified by Autospine software which measured dendritic spine in length and head diameter. Spine length was quantified for all spines of .01-4μm in length. Spines greater than 5μm in length were determined as filopodia and were excluded from the analysis of the data. Spine head diameter of .3-1.2μm were included in the analysis as previously reported (Roscoe, Jr. et al., 2014).

2.6 STATISTICAL ANALYSIS

Data analyses were performed using SPSS version 22 (IBM Corp., Somers, NY) and figures were produced with GraphPad Prism version 5.02 (GraphPad Software, Inc., La Jolla, CA). Animal weights were analyzed with a 2-way mixed model ANOVA with genotype (F344 control vs. HIV-1 Tg) as the between subjects factor, and animal age (PND) as the within subjects factor for analysis. All statistical adjustments for violations of the sphericity assumptions used the most conservative method, Greenhouse-Geisser degrees of freedom correction factor (Greenhouse & Geisser, 1959) and were reported as: p_{GG}. Mauchly's test of sphericity was used to determine violations of the sphericity assumption. An α of $p \leq .05$ was considered statistically significant.

For analysis of Phase I open-field activity measures, a 3-way mixed model ANOVA with genotype (F344 control vs. HIV-1 Tg) as the between-subjects factor with *week* (7 weekly repetitions) and *bin* (12, 5-minute bins) as within-subjects factors was used. Animal gross movements, fine movements and rearing were analyzed. It was hypothesized that significant differences between groups in locomotor activity, similar to previous reports (June et al., 2009; Midde et al., 2011; Moran et al., 2013) would be found in the current study of the HIV-1 Tg rat.

To determine if there was a significant difference between the two genotype groups in diurnal running; Phase II and Phase III running distances were compared through a 2-way mixed model ANOVA, with genotype (F344 control vs. HIV-1 Tg) as the between-subjects factor and *Day* serving as the

within-subjects factor. Running bout length and number of running bouts was analyzed with a 2-way mixed model ANOVA, with identical variables in the analysis. Diurnal and nocturnal running were analyzed separately as it was hypothesized there would be an effect of testing time-of-day manipulations.

Phase IV (Deprivation/Response) running distances were analyzed with a 2-way mixed model ANOVA with genotype (F344 control vs. HIV-1 Tg) as the between subjects factor and *locking duration* (1, 2, or 3 nights locked) as the within subjects factor of the analysis. Running bout frequency was analyzed with Chi-Square Independence tests to determine differences between genotype groups in the frequency of the number of running bouts between stabilized running levels (last week of nocturnal running sessions) and number of running bouts in response to the deprivation of the running wheel.

Diurnal Return analysis was analyzed with a 3-way mixed model ANOVA with *genotype* as the between subjects factor and *week set* (Last week vs. Diurnal Return) and *Day* as within subjects factors in the analysis. Stabilized and Diurnal Return running bout length and frequency were analyzed with a 3-way mixed model ANOVA and analyzed with identical variables. Peak running speed for the groups was analyzed with one-way ANOVA to assess the effect of the HIV-1 genotype on maximum speed attained. For analysis of running initiation latency, a one-way ANOVA was used to compare group latency to initiate running behaviors. Running bout data were analyzed with 2-way mixed model ANOVA, with genotype as the between subjects factor in the analysis, and *Day* as the within subjects factor. In addition, the frequency of the number of running

bouts was analyzed with Chi-Square Independence tests, to examine population changes in the number of running bouts frequency between the two groups. Diurnal and Nocturnal sessions were analyzed separately.

Spine measurements of length and head diameter were analyzed as a population; frequencies of dendritic spine measurements were analyzed through Chi-Square Independence tests to determine any effect of the HIV-1 transgene on dendritic spine length and head diameter (analyzed independently) as a population.

Table 2.1: Experimental Design table

Phase of Study	Phase Breakdown	Fixed Effects	Random effects
Phase I	Weekly Open-Field activity measures (Total 7 weeks)	Fixed effect: Genotype 2 Levels (Control & HIV-1 Tg)	Random Effect: Within-subject variability over 7 trials
Phase II	Diurnal voluntary wheel running until group asymptotic performance for 3 consecutive weeks Total of 5 weeks (35 days)	Fixed effects: Genotype, 2 Levels (Control & HIV-1 Tg) Diurnal Phase 2 Levels (Diurnal & Nocturnal)	Random Effect: Within subject total running distance over course of diurnal running, Within subject latency to initiate running
Phase III	Nocturnal voluntary wheel running until group asymptotic performance for 3 consecutive weeks Total of 8 weeks (56 days)	Fixed Effects: Genotype, 2 Levels (Control & HIV-1 Tg) Nocturnal Phase 2 Levels (Diurnal & Nocturnal)	Random Effect: Within subject variability in total running distance over course of nocturnal running, Within subject latency to initiate running
Phase IV	Nocturnal extinction/reinstatement trials Total of 1.5 weeks (10 days)	Fixed Effects: Genotype, 2 Levels (Control & HIV-1 Tg) Length of extinction, 3 levels (1, 2, or 3 days of extinction)	Random Effect: Within subject variability in total running distance over 3 days of reinstatement, Within subject latency to initiate running
Diurnal Return	Diurnal voluntary wheel running following nocturnal and extinction phases Total of 1 week, (7 days)	Fixed Effects: Genotype, 2 Levels (Control & HIV-1 Tg)	Random Effect: Within subject variability in total running distance over 3 days of reinstatement, Within subject latency to initiate running

Table 2.1: Experimental Design table of each experimental phase of the study. Each phase of the study was run sequentially following the termination of the previous phase.

Table 2.2: Phase IV sequence

Day:	1	2	3	4	5	6	7	8	9
Wheel Status:	**X**	Response recording	**X**	**X**	Response recording	**X**	**X**	**X**	Response recording

Table 2.2: Breakdown of sequential order of Phase IV deprivation/response trials. Each response trial followed a deprivation series, of which the length of the wheel locking was manipulated.

*Note: **X**=Running Wheel locked during nocturnal session.

CHAPTER 3

RESULTS

3.1 BODY WEIGHTS

Animal body weight analysis of the 45 animals which provided weight data for all days of the current study, showed the HIV-1 Tg animals weighed significantly less than their F344 control counterparts across the 192 days of weights analyzed F (1, 43) = 26.899, $p_{GG} \leq .05$, (Figure 3.1). Animal body weights showed a significant effect of Day (i.e. animal age) F (191, 8213) = 959.311, $p_{GG} \leq 0.05$, with a non-significant interaction between Day*Genotype F (191, 8213) =1.512, $p_{GG} > 0.05$.

3.2 LOCOMOTOR ACTIVITY

3.2.1 Gross Movement:

A significant main effect of Day F (6,270) =6.91, $p_{GG} \leq .05$, and Bin F (11, 495) = 619.19, $p_{GG} \leq .05$, and Genotype F (1, 45) =33.66, $p_{GG} \leq .05$ in gross locomotor activity was found (Figure 3.2). Animal Genotype x Bin interactions were significantly different between groups: Bin*Genotype: F (11, 495) = 7.72, $p_{GG} \leq .05$; whereas Genotype*Day was not significant: F (6, 270) =1.191, $p_{GG} > .05$. The 3-way interaction of Bin x Day x Genotype was significant F (66, 2970) = 1.819, $p_{GG} \leq .05$.

3.2.2 Fine Movement:

Results showed a main effect of Day F(6, 270) =27.04, $p \leq .05$; and Bin F(11,495) =693.321, $p_{GG} \leq .05$, and Genotype F(1, 45) =19.50, $p \leq .05$, (Figure 3.3) in animal fine movements in the activity chambers. A significant 2-way interaction was found in the Bin*Genotype interaction F(11, 495) =8.44, $p_{GG} \leq .05$; while the Day*Genotype interaction was not significant F(6, 270) =.268, $p > .05$. The 3-way interaction of Day*Bin*Genotype was not significant for fine movements, F (66, 2970) =1.12, $p_{GG} > .05$.

3.2.3 Rearing:

Results showed a main effect of Day F(6, 270) =46.99, $p \leq .05$; and Bin F (11,495) =543.00, $p_{GG} \leq .05$, as well as Genotype F(1, 45) =19.50, $p \leq .05$. A significant 2-way interaction was found in the Bin*Genotype interaction F(11, 495) =9.09, $p_{GG} \leq .05$; and the Day*Genotype interaction was significant F(6, 270) =.2.63, $p > .05$ (Figure 3.4). The 3-way interaction of Day*Bin*Genotype was not significant for animal rearing, F(66, 2970) =1.06, $p_{GG} > .05$.

3.3 WHEEL RUNNING

3.3.1Diurnal Running

Diurnal running sessions showed a significant difference between days in diurnal running sessions across the 35 consecutive days of diurnal running (5 weeks, 7 days/week) F(34, 1530)=16.41, $p_{GG} \leq 0.05$ (Figure 3.5). However, there was no significant difference between genotype groups in diurnal running

sessions F(1, 45) =0.015, p_{GG} >0.05; nor a significant interaction effect between diurnal running sessions* genotype F(34, 1530) =.999, p_{GG} >0.05.

The diurnal running bout analysis showed a significant effect of running Day F(34, 1530) = 2.925, p_{GG} ≤ .05,no significant difference between groups F(1, 45) = 1.428, p_{GG}>.05, and a non-significant interaction of Genotype*Day F(34, 1530) =.599, p_{GG}>.05. Additionally, a significant effect of Day F(34, 1530) = 5.431, p_{GG}≤.05, a non-significant main effect of Genotype F(1, 45) = p_{GG} >.05, and a non-significant interaction of Day*Genotype F(34, 1530) = p_{GG} >.05 were found in the number of running bouts.

3.3.2 Nocturnal Running

A significant difference between days F(55, 2420) =5.123, p_{GG} ≤0.05; a non-significant difference between genotypic groups F(1, 44) =.031, p_{GG} >.05 in nocturnal running sessions was found. There was a significant interaction between nocturnal running sessions * genotype F(55, 2420) =1.99, p_{GG} ≤0.05 (Figure 3.5).

Analysis of nocturnal running bout length showed a non-significant main effect of Genotype: F (1, 45) = 1.451, p>.05; and Day: F (48, 2160) = 1.566, p_{GG} >.05, the interaction was also non-significant Day*Genotype: F (48, 2160) p_{GG} >.05. However, the analysis of the number of running bouts during the nocturnal sessions showed a significant effect of Genotype F (1, 45) = 5.036, p≤ .05, a significant effect of Day F (48, 2160) = 3.590, p_{GG} ≤ .05, and a non-significant interaction of Day*Genotype F (48, 2160) = .645, p_{GG} >.05 (Figure 3.6).

The number of running bouts of the first 3 weeks of the nocturnal sessions (Weeks 6, 7, & 8) and the subsequent 3 weeks (Weeks 10, 11, & 12) was examined. The analysis showed a significant main effect of genotype $F(1, 44) = 13.933$, $p \leq .05$; a significant effect of "Day" for each set of weeks $F(20, 880) = 4.371$, $p_{GG} \leq .05$; a non-significant effect of the "week set" (Weeks 6,7 8 vs. Weeks 10,11 ,12): $F(1, 44) = 3.742$, $p = .06$; and the Day*Week set interaction was significant $F(20, 880) = 3.635$, $p_{GG} \leq .05$. No other interaction between Genotype or Week set proved significant.

3.3.3 Maximal Speed

Maximal running speed for the groups was determined (F344 Controls *mean*=31.04, *SEM=2.732;* HIV-1 Tg *mean*=32.52, *SEM*=3.129). 1-way ANOVA analysis showed the groups were not significantly different in their maximal running speeds: $F (1, 45) = .129$, $p > .05$.

3.3.4 Latency to Initiate Running

Latency to initiate running behavior was examined. Latency to initiate running was determined by quantifying the time taken (in minutes) to record the first revolution of the running wheel in each nocturnal session. The 1-way ANOVA showed no significant difference in latency to initiate running based on Genotype: F344 Control (*mean=1.745, SEM=.447);* HIV-1 Tg *(mean=1.055, SEM=.447)* $F (1, 45) = 1.171$, $p > .05$.

3.3.5 Deprivation/Response

Deprivation/response data was analyzed to determine the effect of the wheel locking compared to stabilized running distances on the final week of nocturnal running. A 2-way mixed model ANOVA was used for analysis, and showed wheel locking significantly increased the animal's running distance compared to their nocturnal running distances of the previous week: $F(3, 132) = 3.717$, $p \leq .05$, (Table 3.1). Between groups variances were analyzed through mixed model ANOVA, and showed the significant results on wheel locking duration were not due to genotype differences $F(1, 44) = .750$, $p > .05$.The interaction of Response*Genotype was also not significant: $F(3,132) = .379$, $p > .05$. An increase in animal running distances following locking of the wheels suggests the running activity was rewarding for the animals, similar to previously published studies which deprived animals from rewards (Amsel & Roussel, 1952; Heyse, Brenes, & Schwarting, 2015; Kagan & Berkun, 1954).

Additionally, the number of running bouts during stabilized nocturnal running weeks compared to the number of Deprivation/Response running bouts (Figure 3.7) through Chi-Square Independence tests were examined. The analysis showed the relationship between genotype * stabilized week was significant $LX^2(15, N=329) = 40.330$, $p \leq .05$, and the relationship between genotype * Deprivation/Response was significant $LX^2(14, N=141) = 28.890$, $p \leq .05$. The relationship of stabilized week * Deprivation/Response was not significant $LX^2(196, N=141) = 155.238$, $p > .05$. The results suggest both groups significantly increased their running distances and number of running bouts in

response to the locking of the running wheels, suggesting equal rewarding efficacy in both groups.

3.3.6 Diurnal Return

A significant difference between the previous final week of diurnal running distances and the return to diurnal running distances (Figure 3.5, Week 16), LastvsReturn: $F(1, 45)=.126$, $p>.05$ was found, however, a non-significant main effect of Genotype $F(1,45)=.043$, $p>.05$, and a non-significant interaction of LastvsReturn*Genotype, $F(1, 45)=.009$, $p>.05$ was shown. The 3-way interaction of LastvsReturn*Day*Genotype failed to reach significance: $F(6, 270) =.970$, $p>.05$. The only significant results of the analysis were the effect of Day $F(6, 270) =13.707$, $p\leq.05$; and the interaction of LastvsReturn*Day: $F(6, 270) =9.492$, $p\leq.05$. The absence of significant differences between the Phase II and the Diurnal Return running distances showed the increases found during the diurnal/nocturnal cycles were not indicative of a conditioning phenomenon, and are a product of circadian entrainment.

3.4 DENDRITIC SPINE ANALYSIS

A Chi-Square test of independence was performed to analyze the relationship between genotype and dendritic spine measures (See Figures 3.8 and 3.9 for sample of MSN images). The analysis showed Length: $X^2(49, N=177893) = 181.058$, $p\leq.05$ (Figure 3.10), and spine head diameter: $X^2(90,$

N=127681) = 907.530, $p \leq .05$ (Figure 3.11); were significantly different as a population between genotypic groups.

Table 3.1: Deprivation/Response Table of results

Deprivation/ Response Results	**Control % Increase**	**HIV-1 Tg % Increase**	**Number of rats increasing/decreasing running distance compared to previous session**
Response I	11.2%	7.1%	F344 Controls: 16/9 HIV-1 Tg: 13/8
Response II	14.2%	18.2%	F344 Controls: 15/9 HIV-1 Tg: 17/3/**1nc**
Response III	12.0%	16.0%	F344 Control: 11/13/ 1nc HIV-1 Tg: 9/12

Table 3.1: Results of the Deprivation Response series of tests. Notice the deprivation/response was able to motivate all animals to significantly increase their running distances compared to stabilized running sessions, regardless of group membership. Most notable is the number of HIV-1 Tg animals which increased their running distances during the Response following 2 nights of locked wheels, with ~85% increasing their running distances. Additionally, both groups increased their running distances compared to stabilized levels in all levels of deprivation (1-day, 2-day, and 3-day), suggesting the rewarding efficacy of the running wheel is not altered in the HIV-1 Tg animals.

Note: nc = No Change in running distance compared to previous session

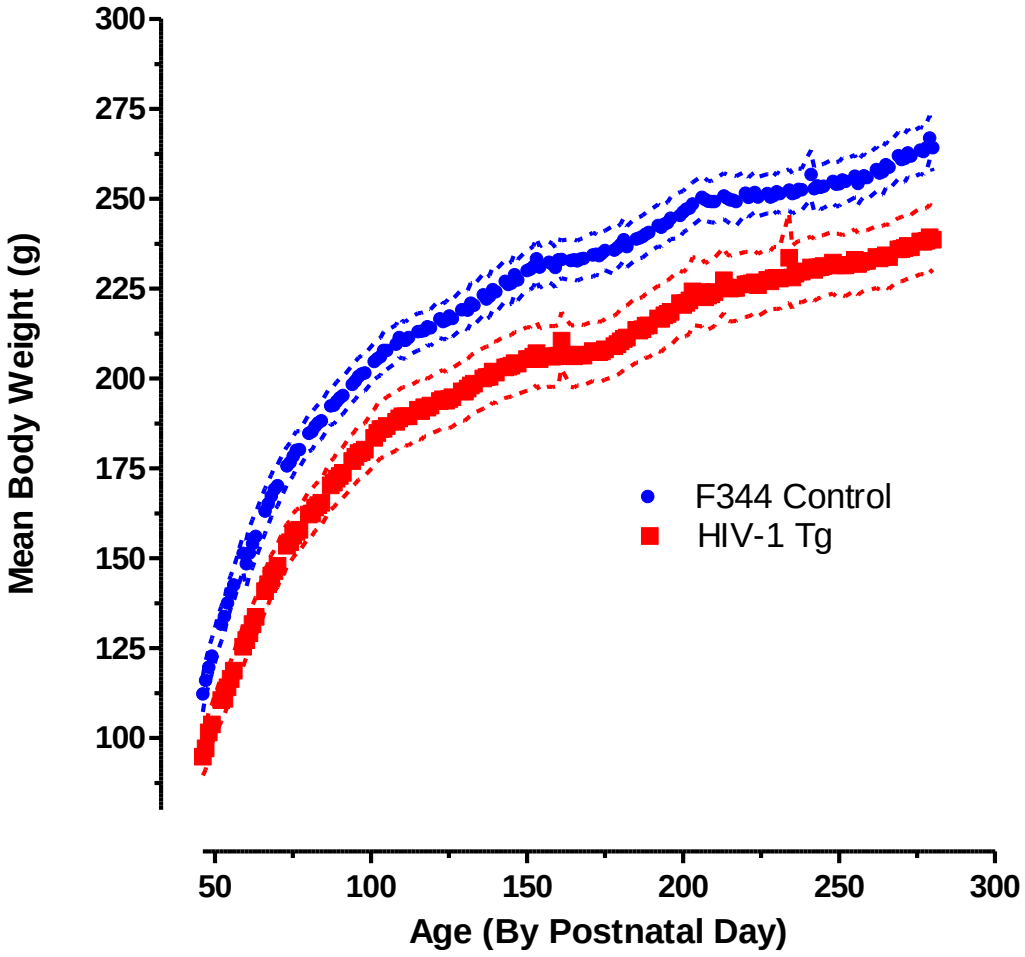

Figure 3.1: Animal body weight means of each group by animal age (Postnatal Day: PND). 95% CIs are plotted. Figure shows the HIV-1 Tg animals grow at the same rate as the F344 controls, but weigh significantly less than their age-matched F344 counterparts. The finding replicates the previously reported differences in body weights in the HIV-1 Tg rat.

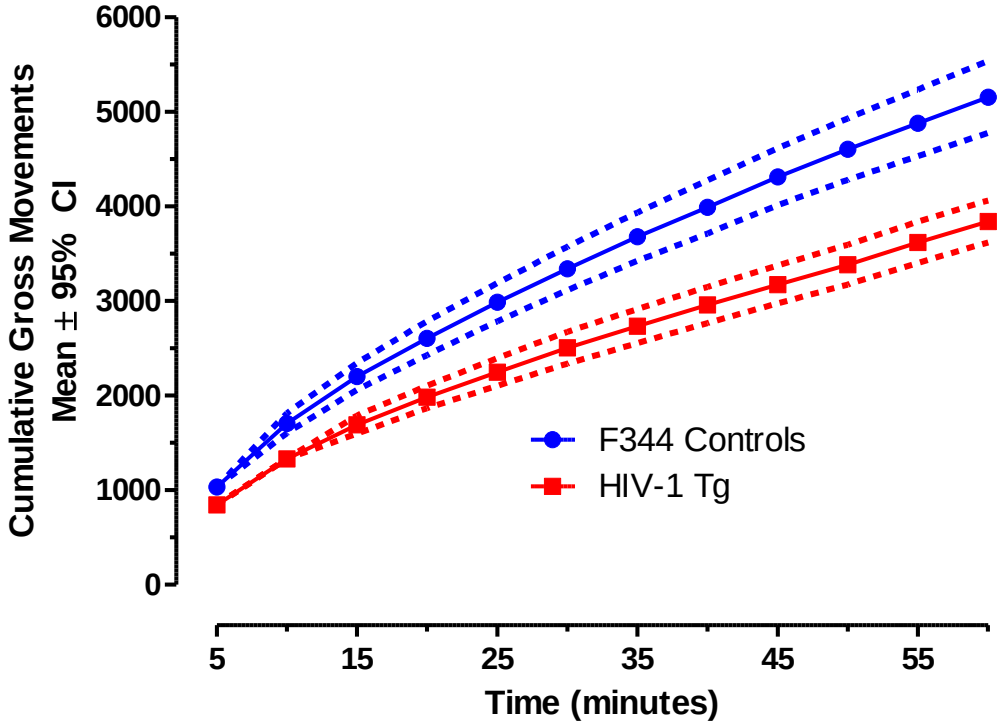

Figure 3.2: Cumulative group mean gross locomotor activity as measured by photocell breaks collapsed across the 7 weeks of locomotor assessment in the activity chambers with 95% CIs. The HIV-1 Tg group showed a significant alteration in their gross locomotor activity habituation over time following similar initial locomotor activity. Cumulative representation of the data shows a reduced, stable trajectory in the HIV-1 Tg rat compared to F344 control's trajectory.

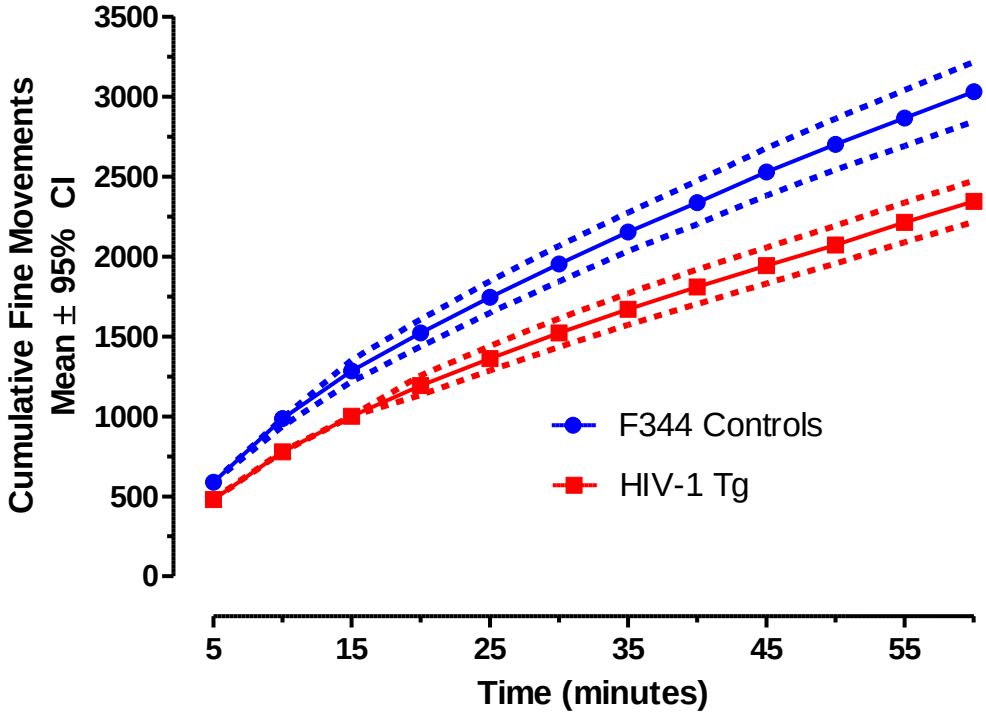

Figure 3.3: Fine locomotor activity cumulative means with 95% CIs for each group, collapsed across the 7 weeks of testing. The graph shows in fine motor activity (head movements, grooming, etc.) the HIV-1 Tg animals show less fine motor activity similar to the results of gross locomotor activity (Figure 3.2) and animal rearing activity (Figure 3.4). Cumulative representation of the data shows a reduced, stable trajectory in the HIV-1 Tg rat compared to F344 controls.

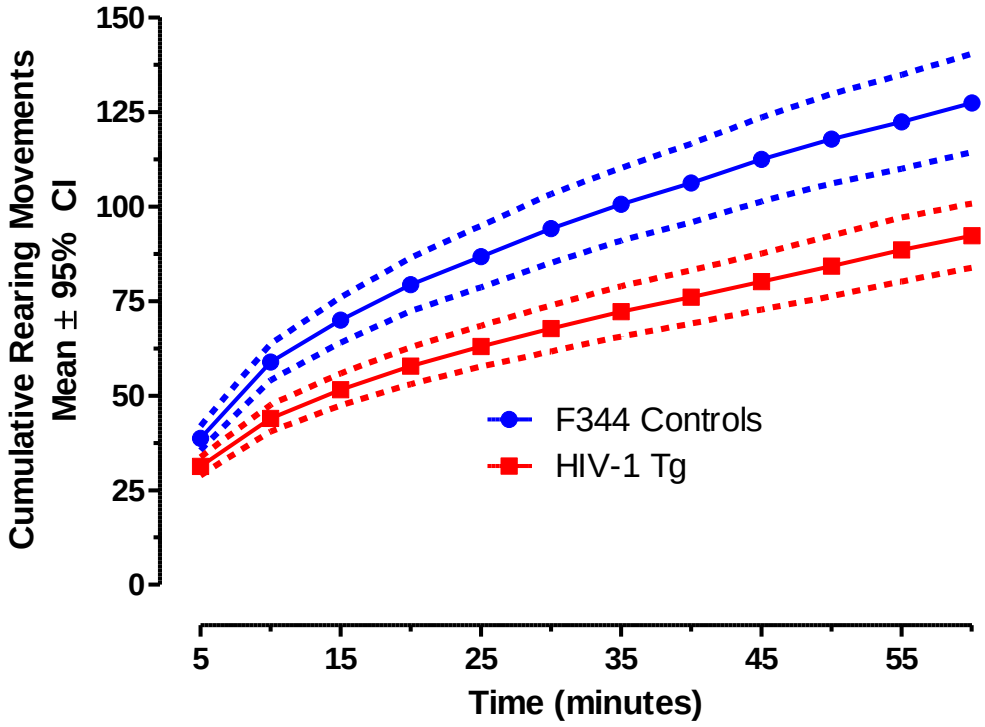

Figure 3.4: Animal rearing (Y-coordinates) cumulative means with 95% CIs, collapsed across the 7 weeks of testing. The HIV-1 Tg animals show less rearing over time compared to the F344 controls. Cumulative representation of the data shows a reduced, stable trajectory in the HIV-1 Tg rat compared to F344 controls.

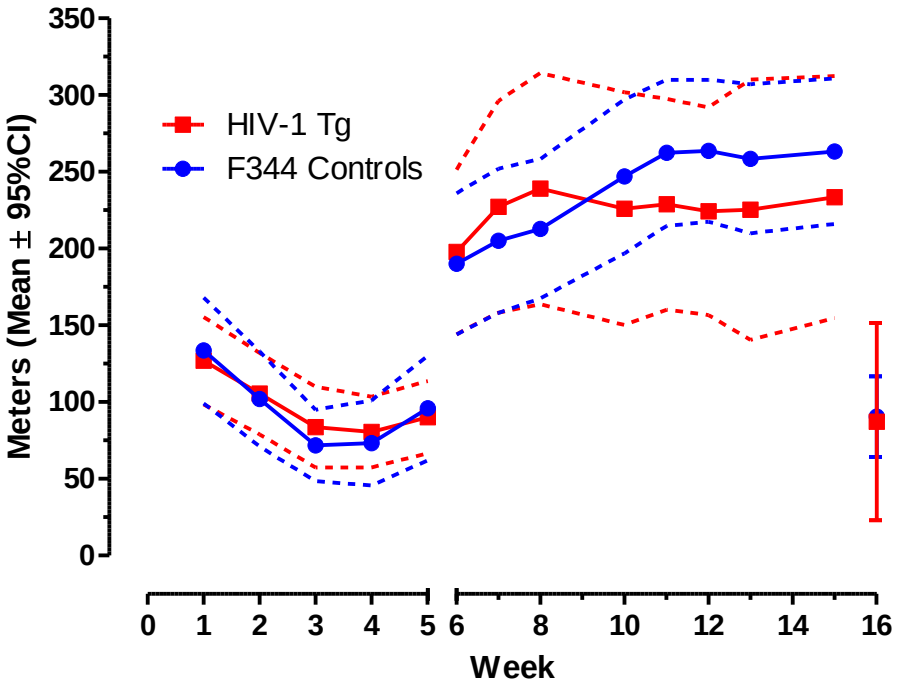

Figure 3.5: Diurnal and Nocturnal mean running distances by group with 95% CIs (Weeks 1-5, and 6-15 respectively). Week 16 (Diurnal Return) was added for clarity. As can be seen, the HIV-1 animals showed an earlier plateau in the overall running distance around week 8, meanwhile the F344 controls continued to escalate until reaching their plateau around week 11 where they stabilized for 3 consecutive weeks. No discernible differences were found in diurnal sessions (Weeks 1-5 & 16).

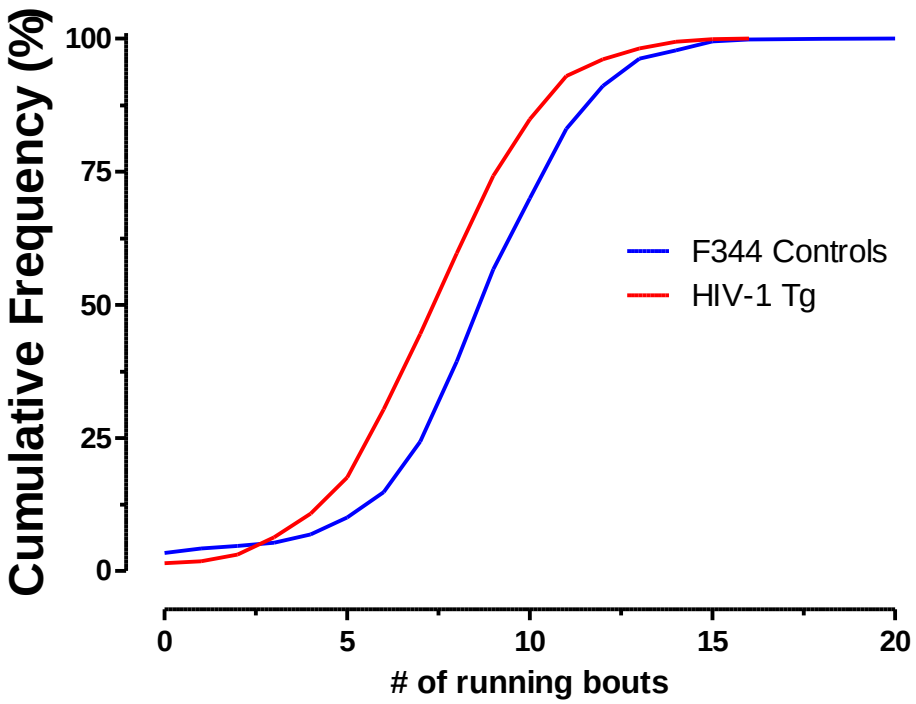

Figure 3.6: Representation of the number of Nocturnal running bouts. The figure shows the reduction in the number of nocturnal running bouts as a population in the HIV-1 Tg animals compared to controls. As a population, the HIV-1 Tg animals show a significant reduction in the number of running bouts per nocturnal session ($F(1, 45) = 5.036$, $p \leq .05$) compared to controls.

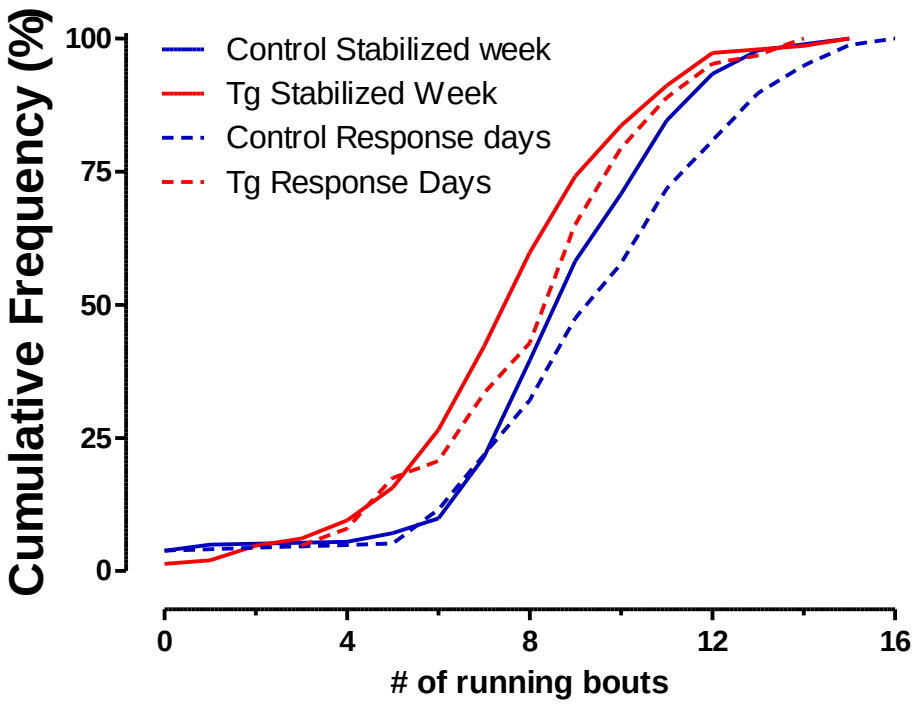

Figure 3.7: Representation of the number of Nocturnal running bouts, in both the stabilized week of nocturnal running sessions and the animal's response to wheel deprivation. The figure shows the reduction in the number of nocturnal running bouts as a population in the HIV-1 Tg animals compared to controls; and the finding was repeated during the response phase following wheel deprivation. As a population, the HIV-1 Tg animals show a significant reduction in the number of running bouts per nocturnal session in their stabilized weeks: $LX^2(15, N=329) = 40.330$, $p \leq .05$, which carried over to the Response phase of the study: $LX^2(14, N=141) = 28.890$, $p \leq .05$.

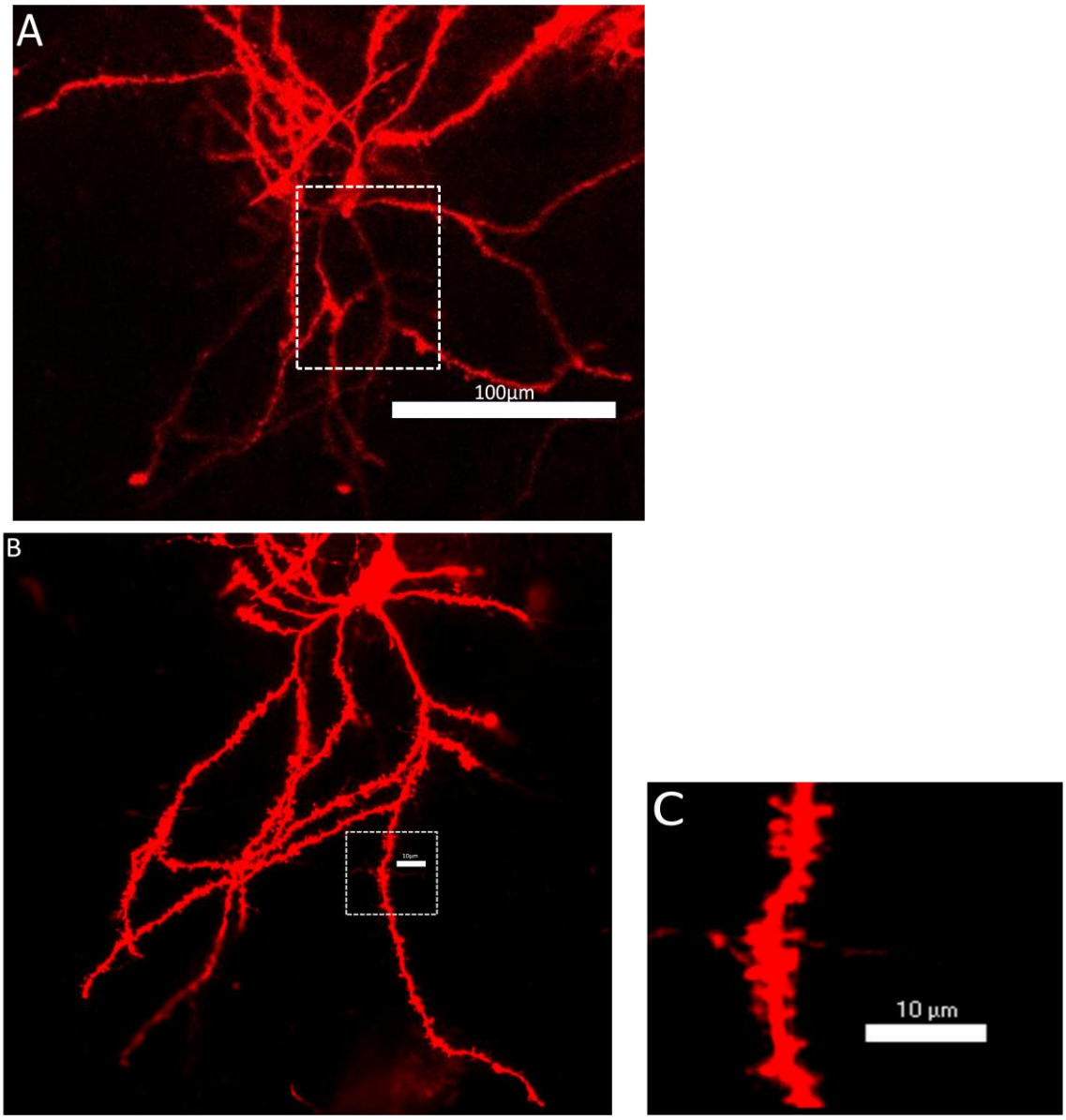

Figure 3.8: Sample images from F344 control (animal #39). **(A)** 20x image of MSN, **(B)**60x image of same MSN, **(C)**zoomed 60x image of MSN.

*Note: Dashed boxes are the area examined in the subsequent picture.

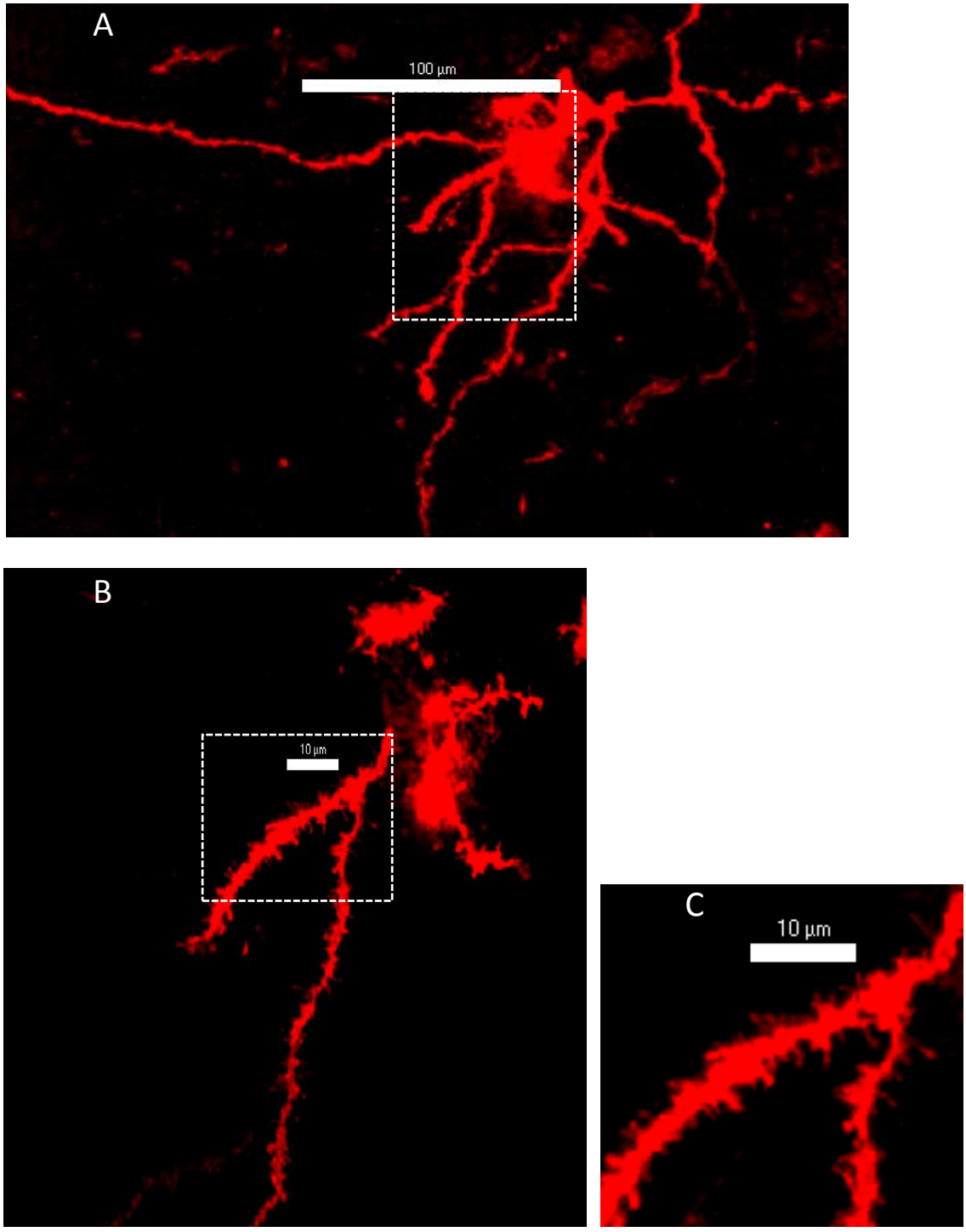

Figure 3.9: Sample images from HIV-1 Tg animals (animal #40). **(A)**20x image of MSN, **(B)**60x image of the same MSN, **(C)**=zoomed 60x image of MSN.

*Note: Dashed boxes are the area examined in the subsequent picture

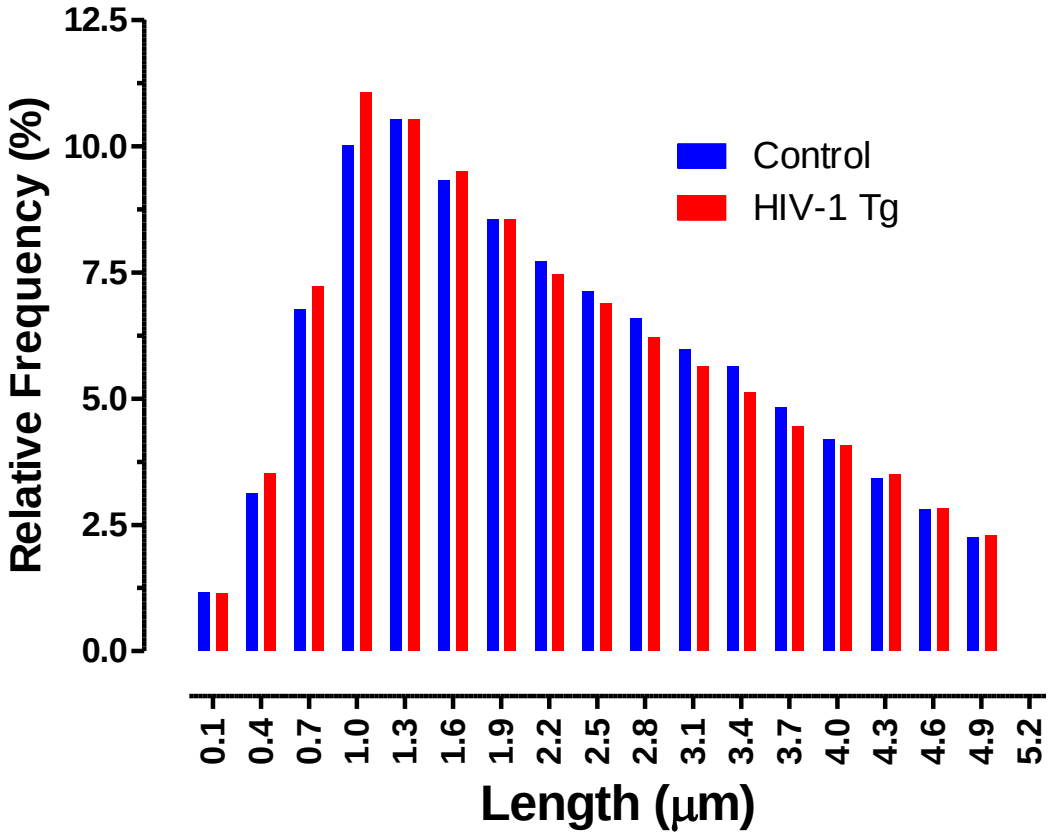

Figure 3.10: Figure of NAc MSN dendritic spine length frequencies. The HIV-1 Tg rats as a population showed significantly shorter NAc MSN dendritic spines: $X^2(49, N=177893) = 181.058, p \leq .05$).

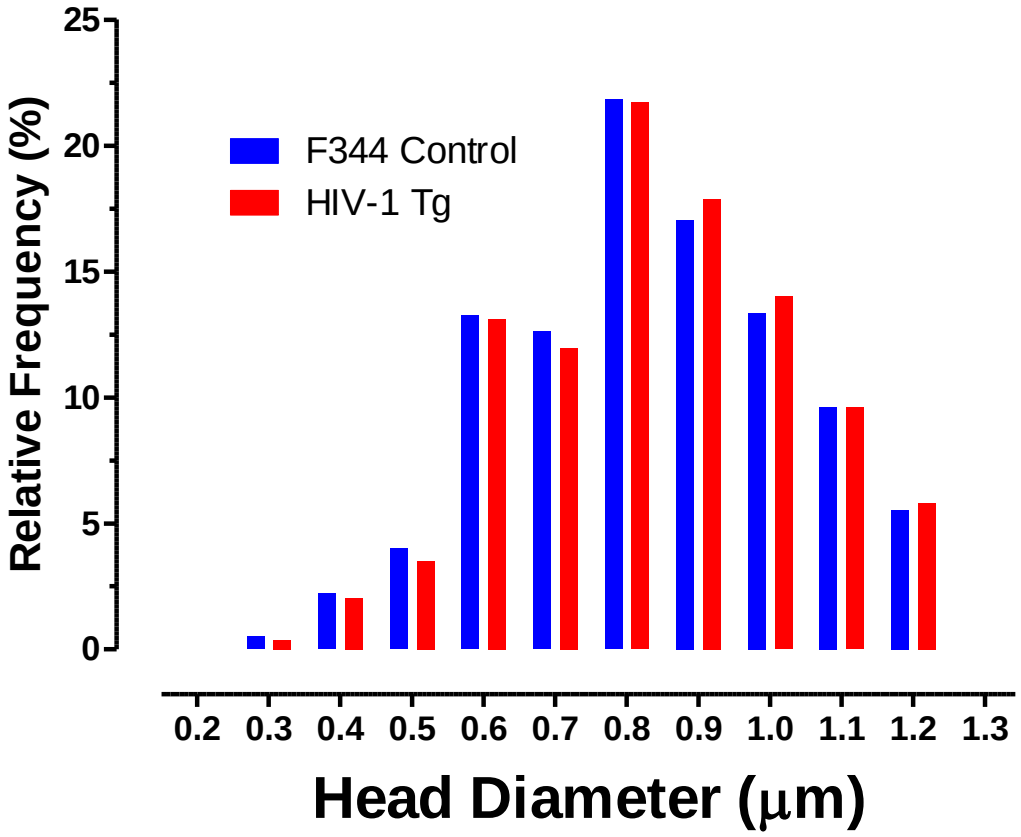

Figure 3.11: Figure of NAc MSN dendritic spine head diameter frequencies. The HIV-1 Tg rats as a population showed significantly wider NAc MSN dendritic spines: $X^2(90, N=127681) = 907.530$, $p \leq .05$.

CHAPTER 4

DISCUSSION

The motivation of the HIV-1 Tg rat, as well as circadian influences on voluntary wheel running were examined by the current study. Additionally, examination of the dendritic spines of the MSNs found in the NAc were compared. The resulting measurements were compared between the two groups as a population. Motivational differences in the HIV-1 Tg rat during the nocturnal phase of the voluntary running activity compared to F344 controls were found. The motivational differences in the HIV-1 Tg rat were observed and operationalized through reduced running distances and a reduction in the number of running bouts. These results were dependent upon circadian influences, as no differences were found during diurnal testing sessions. Furthermore, a potential mechanism for the reduction in motivation in the HIV-1 Tg rat, through MSN dendritic spine alterations was shown.

A motivational deficit in the HIV-1 Tg rat for naturally-rewarding voluntary wheel running compared to age matched controls was established by the current study. HIV-1 individuals are subject to feelings of apathy, presumable caused by basal ganglia atrophy (McIntosh et al., 2015; Paul et al., 2005), and neural alterations from HIV-1 viral proteins. Behaviorally, an increase was shown in the HIV-1 Tg rat's nocturnal running distances during their peak activity hours

(Goff & Finger, 1966; Peng, Jiang, & Hsu, 1980; Shirley M., 1928b). An earlier plateau in running distances in the HIV-1 Tg group was shown by the results, suggesting a maximal level of rewarding efficacy was reached earlier in the HIV-1 Tg rat. In the present study, it may be argued the reduction in dendritic spine length and increased head diameter to be potential mechanisms for the reduced running distances and number of running bouts in the HIV-1 Tg animals. Additionally, perhaps the reductions in running distances and running bouts were due to dopamine transporter (DAT) dysregulation (McIntosh, Sexton, Pattison, Childers, & Hemby, 2015; Midde, Gomez, & Zhu, 2012), thereby affecting the motivational for a naturally rewarding stimuli. The animals did not show differential maximal speed, nor an increased latency to initiate their first running revolution, or an alteration in the rewarding properties of the running wheel was shown by the running wheel data. Instead, a reduced “desire” or motivation to perform a naturally rewarding activity consistently, operationalized through reduced running distances and reduced number of running bouts in the HIV-1 Tg rats compared to controls was found.

As rats are most active during nocturnal hours (Goff & Finger, 1966; Peng et al., 1980; Shirley M., 1928b), the reduction in motivation indicated by the decreased running distances and running bouts was found strictly during the animal’s most active hours, and not impaired during diurnal sessions compared to controls. It was also demonstrated with the Diurnal Return data, that the differences in diurnal and nocturnal running distances were not a product of conditioning, but rather, an example of entrainment to a natural light/dark cycle.

The well-entrained circadian cycle suggests the HIV-1 Tg rat does not exhibit circadian alterations in a motivated behavior and was capable of adjusting to experimental time-of-day manipulations equal to controls. No deficits in the rewarding nature of the running activity was shown by the deprivation/response tests (Mueller et al., 1999) in this sample, but rather a deficiency in the long-term maintenance and/or escalation of a naturally rewarding activity compared to age-matched controls during nocturnal sessions. Nearly equal population shifts in the number of running bouts per session suggests the motivation for reward following deprivation is reasonably intact in the HIV-1 Tg rat, which was shown by the animals in this sample. However, it should be noted an altered running bout shift was shown by the increases in the bout number between the groups, i.e. the increase in number of running bouts was greater in the more active controls (top 50%) compared to the more active HIV-1 Tg animals (top 50%).

One of the most striking and informative findings in the study was the results of the Diurnal Return phase of the experiment. It was initially hypothesized that returning the animals to the diurnal schedule would result in the animals running a distance somewhere between their previously established diurnal running and their stabilized nocturnal running distances as a product of *conditioning*. However, the animals returned to the previously stabilized diurnal distances. Thereby suggesting the differences seen in the transition from diurnal to nocturnal running were completely entrained to the animal's circadian cycles and were robust to withstand prolonged manipulations in testing time-of-day. Circadian activity entrainment is incredibly robust and difficult to dramatically

uncouple from the natural light/dark schedule beyond a few hours with light/dark schedule manipulations (Goff & Finger, 1966). In the present study, time-of-day manipulations were resisted by the HIV-1 Tg animals, which followed their circadian schedule of activity equally as well as controls. The Diurnal Return findings are in agreement with the work of Duncan et al. (2008) which found normal circadian cycles (albeit reduced circadian amplitudes) in mice with neural Tat expression via doxycycline promoters, suggesting the HIV-1 Tat protein is not the mechanism of circadian alterations found in HIV-1 seropositive patients.

The possibility the results were due to a latency to initiate running bouts was assessed. Motor behavior initiation is a hallmark of Parkinson's disease, which is a product of dopaminergic basal ganglia alterations (Contreras-Vidal & Stelmach, 1995). The effects of the dopamine-targeting HIV-1 transgene causing motor initiation disabilities (Graybiel, 1990; Growdon, Kieburtz, McDermott, Panisset, & Friedman, 1998) in the HIV-1 Tg animals and thereby reducing their overall running distances was examined. Motor initiation deficits were not apparent, as suggested by the latency results, as the groups showed no significant difference in their initiation of their first running bout in nocturnal sessions. Animal nocturnal running bout length and frequency were also examined. A significant reduction in the number of running bouts in the HIV-1 Tg animals was found, however, no statistically significant differences were found in running bout length. This finding was attributed as a manifestation of reduced motivation for the naturally rewarding running behavior.

The finding of reduced running distances in the HIV-1 Tg animals is in opposition to that previously reported (Chang & Vigorito, 2006), which found the HIV-1 Tg rat ran *greater* distances than weight-matched F344 controls. However, the Chang and Vigorito (2006) study differed in total animals used (10 vs. 47), animal sex (males vs. ovariectomized females), running session length (30minutes vs. 60 minutes), the number of daily diurnal running repetitions (15 vs.35), time-of-day (diurnal vs. diurnal & nocturnal) and weight-matched vs. age-matched animals. Differential running distances based upon animal age (Peng et al., 1980) were shown in animals of different ages. As such, compared to weight-matched groups, superior assessment of motivation for wheel running is provided by age-matched groups.

The HIV-1 Tg animals displayed structural alterations of their MSNs in the NAc as shown by the dendritic spine data. A potential mechanism of reduced motivation in the HIV-1 Tg rat was provided by the finding of significantly reduced dendritic spine length and increased spine head diameter. As activation of the mesocorticolimbic pathway is essential for motivated, naturally rewarding behaviors (Basso & Morrell, 2015; Greenwood et al., 2011; Mogenson et al., 1979; Mogenson & Yang, 1991; Pijnenburg et al., 1976; Rhodes et al., 2005; Roberts et al., 2012), motivational alterations may be produced by alterations of the MSNs of the mesocorticolimbic pathway. Collectively, the behavioral analysis coupled with the histological data suggests it may be the case.

Alterations of the dendritic spines as a population compared to controls suggests synaptodendric injury or alterations in the structural proteins of dendritic

spines are occurring in the HIV-1 Tg rat. Altered NAc MSN dendritic spines potentially led to the reductions in motivation found in the data of the HIV-1 Tg rat, which was assessed through reduced wheel running distances and reductions in the number of running bouts. A potential mechanism of the increase in immature dendritic spines (shorter and stubbier) in the HIV-1 Tg rats may be alterations of the *GTPases* related to the actin assembly of dendritic spines. Multiple mechanisms are responsible for dendritic alteration in dendritic plasticity spine growth and morphological changes, one of which is the small GTPases: Ras-related C3 botulinum toxin substrate 1 (Rac1) and Ras homolog gene family, member A (RhoA) (Lai & Ip, 2013). Rac1 is responsible for spine growth via activation of the PAK1 and LIMK1 while RhoA regulates and inhibits spine formation and stabilization. Coincidentally, the Tat protein has been shown to activate the RhoA signaling pathway *in vitro* (Krogh, Lyddon, & Thayer, 2015). Upregulation of the RhoA pathway *in vivo* from chronic Tat exposure would theoretically keep spines in a state of immaturity resulting in reduced synaptic efficiency. Further insight into the neurobiological alterations in the HIV-1 Tg rat was provided by the histological examination of the MSNs of the HIV-1 Tg rats. The dendritic spines of the MSNs of the NAc were significantly different as a population in the HIV-1 Tg animals compared to controls, thus providing evidence the mesocorticolimbic pathway is altered in the HIV-1 Tg animals, which may be due to RhoA upregulation by the Tat protein which is essential for motivated behaviors.

Furthermore, AMPA/NMDA receptors are formed during plasticity-dependent alterations in the MSNs of the NAc. Activation of the AMPA receptor in glutamatergic synapses (such as MSNs) is required in long-term potentiation (LTP). Both AMPA and NMDA receptors are able to bind glutamate in normal tonic activation and allow Na^+ to flow inside of the postsynaptic membrane through the AMPA receptor exclusively. Such is the case during low-frequency EPSPs(i.e. weak stimulation) from the presynaptic neuron. Na^+ only flows through the AMPA receptor, as the NMDA receptor has a Mg^{2+} molecule resting within the NMDA channel. During high-frequency EPSPs, the voltage-gated NMDA channel changes confirmation, and forces the Mg^{2+} block out of the channel to allow Na^+ and Ca^{++} to flow into the postsynaptic neuron. The influx of Ca^{++} initiates intracelluar signaling cascades such as Ca^{2+}/calmodulin-dependent protein kinase (CaMKII) and protein kinase C (PKC) which induces LTP. Continuous low-frequency EPSPs cause a slow, steady increase in Ca^{++} in the postsynaptic neuron, which leads to long-term depression (LTD) which reduces the synaptic contacts and reduces the number of AMPA receptors. Alternatively, fast and large increases in Ca^{++} promote increased AMPA receptor expression and strengthen synaptic connections. During LTP the sensitivity of the postsynaptic cell to glutamate is increased through expression of additional AMPA receptors. Which thereby increase the ability of higher frequency EPSPs to remove the Mg^{2+} block from the NMDA receptor, thus yielding a strengthening of the synapse (Purves et al., 2012c). Alterations in synaptic creation/modification (dendritic spines) from HIV-1 viral proteins may therefore

affect the efficacy of rewarding stimuli. In the current study, the histological data suggests the differences found in a naturally rewarding behavior (wheel running) may be a product of synaptic alterations and reduced plasticity-dependent efficiency. Previous studies have shown HIV-1 Tat is able to interact with the NMDA receptors of the hippocampus and produce learning deficiencies (Self, Smith, Butler, Pauly, & Prendergast, 2009), thus it is possible alterations in motivation may be created by altered MSN plasticity.

Ovariectomized females were chosen for the current study because worldwide, HIV-1 infection afflicts women slightly more than men, comprising ~51% of all cases (World Health Organization, 2015), and women are at an increased risk for neurocognitive deficits compared to men (Chiesi et al., 1996). Furthermore, women are underrepresented in the scientific literature in neuropsychological studies where most studies contain only male subjects or male rats (Fox-Tierney, Ickovics, Cerreta, & Ethier, 1999). The animals were ovariectomized, as wheel running follows an estrus cycle-dependent pattern (Anantharaman-Barr & Decombaz, 1989) in intact female rats. Additionally, it has been shown at least in cell cultures that 17β-estradiol is capable of reducing apoptosis from the HIV-1 Tat protein (Adams, Aksenova, Aksenov, Mactutus, & Booze, 2010). Collectively, these studies warrant the examination of HIV-1 neuroprogression in ovariectomized female animals in the current study.

Outside of dendritic spine alterations in the HIV-1 Tg rat, decreased running activity may also be due to decreased dopamine efficiency in the HIV-1 Tg animal, based on previous work which examined the dopaminergic

projections from the VTA and NAc (Greenwood et al., 2011; Mogenson et al., 1979; Mogenson & Yang, 1991; Pijnenburg et al., 1976; Roberts et al., 2012) and their necessity for motivated behaviors such as area exploration and wheel running. The reduction in dopaminergic efficiency may be caused by DAT dysregulation which is found in the HIV-1 Tg rat (McIntosh et al., 2015; Midde et al., 2012) as well as in HIV-1 seropositive humans (Chang et al., 2008; Wang et al., 2004). The DAT is directly affected by the HIV-1 protein Tat (Midde et al., 2012; Midde et al., 2013; Yuan et al., 2015; Zhu, Mactutus, Wallace, & Booze, 2009). Deficits in the DAT would produce behavioral alterations in motivated behaviors which require dopaminergic activation, such as wheel running and area exploration. The HIV-1 Tg rat ran lower distances and produced fewer running bouts as a population compared to controls as shown by the data, which is indicative of mesocorticolimbic dopaminergic dysregulation. This hypothesis is supported by the histological findings of the NAc MSN spine alterations. Additionally, the motivational efficacy for wheel running was not altered in the HIV-1 Tg rat compared to F344 controls as shown by the results of the Deprivation/Response data, as both groups escalated their running distances in response to the deprivation of the running wheel, albeit at slightly different rates. Running is a rewarding activity, as shown by the deprivation/response trials, as both groups of animals increased their running activity compared to previously stabilized levels. An increase in responding is indicative of mesocorticolimbic activity (Basso & Morrell, 2015; Mogenson et al., 1979; Mogenson & Yang, 1991; Pijnenburg et al., 1976) regarding a rewarding stimulus. Interestingly, the HIV-1

Tg animals took 2 days of deprivation to produce the equivalent increase in running distances compared to controls.

Significant differences in body weight was shown in the HIV-1 Tg animals, similar to previous studies (June et al., 2009; Midde et al., 2011; Moran et al., 2013). The animals weighed significantly less as a group, but importantly, grew at the same rates (not significant Day*Genotype interaction). The body weight analysis suggests the HIV-1 Tg animals are as healthy as controls in their physiological development, and therefore are physiologically capable of running distances equal to age-matched F344 controls. To verify, maximal running speed attained in each group was compared. As the HIV-1 Tg animals are significantly smaller, it is reasonable to theorize they lacked the capacity for running as fast as their control counterparts based on physiological demands, and perhaps is the reason for the differential distance findings. However, the groups were not significantly different in their maximal running speed attained was shown in the Maximal Speed data. Additionally, previous findings (June et al., 2009; Midde et al., 2011; Moran et al., 2013) in the HIV-1 Tg rat regarding motivational deficits in the open-field activity chambers when tested during the diurnal phase of their circadian cycle; as well as altered rates of habituation in all aspects of general motility (gross movements, fine movements, and rearing) was replicated in the current study. The groups showed significantly different rates of habituation in the activity chambers as assessed by the significant genotype × bin interaction. It may be argued the differences in locomotor activity were a behavioral manifestation of apathy in the HIV-1 Tg rat.

However, there are several caveats one must remember when interpreting the results. Primarily, only the motivational properties of the HIV-1 Tg rat was examined, and therefore the interpretation to human seropositive individuals and motivational parallels must be cautious. Secondly, the HIV-1 Tg rat is a genetically altered vehicle for HIV-1 study, created through embryonic alterations, and does not provide information identical to that found in HIV-1 seropositive human subjects, nor does it allow the examination of the fully functioning (9 of 9 genes expressed) HIV-1 virus. Third, the current study only examined a motivated behavior during 1 hour in a 24-hour cycle; what occurred outside of testing hours remains to be determined. However, extrapolation of overall daily activity can be determined through examinations of optimal one-hour testing (Shirley M., 1928a). Finally, caution must be used when interpreting the results to intact females and males as ovariectomized females were utilized in the current study.

In summary the data argue the HIV-1 Tg rat is a potential candidate to examine the motivational deficiencies found in the human HIV-1+ populations, specifically reduced motivation for a naturally rewarding behavior (such as medication adherence). Additionally, a potential mechanism for the reduction in motivation: MSN dendritic spine alterations potentially through upregulation of RhoA signalling by the Tat protein has been suggested. Potential therapeutics can utilize the HIV-1 Tg rat model to establish pharmaceutical therapies to combat the apathy problem in the HIV-1+ human population by creating therapies capable of protecting or restoring the MSNs of the nucleus accumbens.

Reductions of apathy prevalence in the HIV-1 populations may increase medication adherence; thus preventing viral rebound and medication resistance, and improve individual state of mind; with less depressive qualities and improved outlook, collectively resulting in a healthier afflicted individual overall.

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